THE EFFECT OF MALEIC HYDRAZIDE ON THE EMBRYONIC AND LARVAL GROWTH OF THREE AMPHIBIANS

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Since Schoene and Hoffman (1949) first reported that maleic hydrazide (MH) inhibits plant growth, many investigators have extended and in general substantiated their findings. Greulach and Atchison (1950) reported that MH inhibits mitosis in onion root tips, Compton (1951) that it inhibits mitosis in pea seedlings, Girolami (1951) that it inhibits mitosis in flax, and Greulach (1952) that it inhibits mitosis in bean buds. Darlington and McLeish (1951) found that MH inhibited mitosis in the roots of several species of plants, and that it induced chromosome breakage in *Vicia faba,* but not in *Rhoeo discolor, Muscari palmosus,* or *Scilla sibirica.* The breakage was confined to the heterochromatin (instead of to the euchromatin as with X-rays), and no sticky chromosome surface was produced.

Since MH has a striking effect on cell division and growth of plants it would be of interest and value to learn whether MH has similar effects on animals. There is very little published information on the effect of MH on animals. Zukel (1951) reports that the oral LD 50 of MH to rats is 2.2 gm./kg. body weight, and that there is no irritation to the skin or mucous membranes of experimental animals from contact with MH. An earlier mimeographed release from the Naugatuck Chemical Company (producers of MH) stated that acute toxicity of MH to mice by intraperitoneal injection was 1.5 gm./kg. of body weight. Johnson, working at the University of North Carolina, has unpublished data which indicate that MH does not affect the growth of mice nor the growth of testicular tumors in mice. Young white mice sprayed daily for three weeks with six different MH concentrations ranging from 0.001 to 0.2 per cent, or given drinking water with the same concentrations of MH for three weeks, grew as rapidly as the controls and exhibited no toxic symptoms. Older white mice into which testicular tumors were transplanted were injected subcutaneously for ten days with 0.1 ml. of one of nine MH concentrations ranging from 0.05 per cent to the undiluted original solution containing 30 per cent MH by weight. No concentration of MH inhibited growth of the tumors, nor did any concentration produce symptoms of toxicity.

Experiment with Rana pipiens

Ovulation was induced in female frogs (*Rana pipiens*) by pituitary injections, and the eggs were fertilized by sperm from testes macerated in Ringer's solution. Two days later, when the embryos were in the neural tube stage, 15 embryos were placed in boiled and cooled tap water, 15 in a 0.1 per cent solution of MH, and 15 in a 0.05

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per cent solution of MH. The MH was used in the form of the diethanolamine salt and was dissolved in boiled and cooled tap water, the same being true of the other experiments reported here. After 48 hours the embryos were transferred from the MH solutions to tap water and the embryological and larval development were observed over a period of six weeks.

Five days after the beginning of treatments the mean overall length was 10.5 mm. for the controls and both treated groups. The length of the body without the tail was 3.9 mm. for the controls, and 3.6 mm. for both treated groups. The body width was 3.2 mm. for the controls, 2.5 mm. for those treated with 0.05 per cent MH, and 2.4 mm. for those treated with 0.05 per cent MH, and 2.4 mm. for those treated with 0.1 per cent MH. These differences were not statistically significant. There was no observable effect of 0.05 per cent MH, but in the 0.1 per cent MH treatment there were retardation of eye development, smaller myotomes and gills, and a more sluggish response to light and touch than in the controls. Circulation as observed under a binocular dissecting microscope was apparently normal in both treated groups. The mortality rate in the 0.1 per cent MH was slightly higher than in the other two groups, but in the survivors the early minor differences in growth rates were overcome by the end of the experiment.

Experiment With Bufo sp.

Freshly laid eggs of an unidentified species of toad were collected in the field, and 75 embryos in the early blastula stages (with ectodermal epiboly incomplete) were selected and placed in 0.02 per cent MH, 75 similar embryos being placed in boiled tap water. After 48 hours the embryos in MH were transferred to tap water. Five days later four larvae from each group were selected for tail tip amputation and study of the rate and amount of tail tip regeneration, the remainder being retained for observations of growth and development.

The MH had no observable effect on embryonic or larval development, reflexes, or amount and rate of tail tip regeneration.

Experiments With Amblystoma punctatum

Embryos of the salamander, *Amblystoma punctatum,* were obtained in early cleavage to yolk plug stages. The jelly was removed and batches of embryos were placed in tap water which had been standing 48 hours, and in 0.5, 1.0, and 2.0 per cent solutions of MH. After 10 days all the gastrulae were transferred to standing tap water and their development was observed over a period of several weeks. In a second experiment the posterior third of the tail was amputated from a group of larvae 5 days after hatching. The larvae were divided into three equal groups and placed in tap water, 0.25 per cent MH, or 1.0 per cent MH. After 10 days the regenerated tail tips were clipped and then fixed and stained by the Costello-Henley (1949) method. The regenerated tissue was examined under oil for possible effects of the treatment on mitosis, and the number of mitotic figures per tail tip was determined, there being 8 control tail tips and an equal number in each of the two treated groups.

In the first experiment all the embryos in the 2.0 per cent MH died within 50 hours, but 35 per cent of those in the 1.0 per cent MH and 70 per cent of those

in the 0.5 per cent MH survived through the 10 day treatment period. Since only 84 per cent of the controls survived, the 0.5 per cent MH was apparently not particularly toxic. There was no superficial morphological difference between the controls and the surviving treated embryos, nor any significant difference in size. In the second experiment there was no significant difference in the size of the regenerated tail tips in the various groups. The mean number of mitotic figures per regenerated tail tip was 8.5 in the controls, 2.6 in the 0.25 per cent MH, and 3.0 in the 1.0 per cent MH. The differences between both treated groups and the controls were statistically significant at the 1 per cent level, but the two treated groups did not differ significantly from each other. There were many pyknotic nuclei in both treated groups, but none in the controls. All concentrations of MH used promptly killed the Daphnia and Cyclops introduced into the cultures for use as food by the salamander larvae.

Discussion

The results of these experiments indicate that MH does not have any significant or persistent effects on the growth and development of amphibian embryos and larvae similar to its effects on the growth of plants, even though several of the concentrations used on Amblystoma were much in excess of the concentration range of 0.02 per cent to 0.2 per cent usually used on plants.

In these experiments all concentrations of MH used were lethal to Daphnia and Cyclops, the 2.0 per cent MH was lethal to Amblystoma, and the 1.0 per cent MH was toxic to Amblystoma. The 0.1 per cent MH was somewhat toxic to Rana. Except for the latter concentration, all these concentrations were much in excess of those commonly used on plants. Taken in conjunction with the toxicity studies on rats and mice reviewed in the introduction to this paper, these data indicate that MH is toxic to animals only in rather high concentrations, considerably in excess of those used on plants. However, this does not take into consideration possible cumulative or delayed toxicity symptoms of lower concentrations. The sluggishness in some of the treated larvae of both Rana and Amblystoma could possibly be due to a decrease in the rate of respiration, since there is evidence that MH may decrease the rate of respiration in plants.

The data from the second experiment with Amblystoma would seem to indicate that MH did reduce the rate of mitosis in the regenerating tail tips. However, in the absence of any significant effects on total growth, in view of the fact that the two different MH concentrations had no different effect on the number of mitotic figures, and since starvation is known to induce pyknosis and inhibit mitosis in regenerating tail tips, the writers are of the opinion that the reduction of mitosis was not due to a direct effect of MH as in plants, but to starvation resulting from the lethal effect of MH on the crustaceans used by the Amblystoma larvae as food. Amblystoma larvae ingest only live, moving prey. It is planned to repeat this experiment with the addition of a starvation control group and with a wider range of MH concentrations, but in view of the basic complication involved it may be necessary to use other animals and techniques to clarify completely the effect of MH on mitosis in animals.

Summary

1. A 2.0 per cent solution of maleic hydrazide was lethal to all *Amblystoma punctatum* larvae within 50 hours, but 35 per cent of the larvae survived 10 days in 1.0 per cent MH and 70 per cent in 0.5 per cent MH, while 84 per cent of the controls survived. All concentrations of MH from 0.25 per cent upward killed Daphnia and Cyclops introduced into the Amblystoma cultures as food. A 0.1 per cent solution of MH was slightly toxic to *Rana pipiens* larvae.

2. MH concentrations ranging from 0.02 per cent to 2.0 per cent had no significant effect on the growth of embryos and larvae of Rana, Bufo and Amblystoma. The full range of concentrations was not used on any one of the three species.

3. The number of mitotic figures in regenerating tail tips of Amblystoma was significantly less in those treated with either 0.25 per cent or 1.0 per cent MH than in the controls. Pyknosis was common in the treated tail tips but was absent in the controls. The writers are of the opinion that these are indirect, rather than direct effects of the MH treatment, and can be attributed to starvation following the death of the Cyclops and Daphnia provided as food for the Amblystoma larvae.

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